form of a tube. This tube persists until the later stages of development, and grows in length with the whole cervical region without increasing in circumference, so that it finally constitutes a long, thin, caudally directed, cervical, fistulous canal. In young developmental stages of snakes and lizards we certainly meet with a similar canal in a rudimentary condition; in these forms, however, it undergoes no further development, but disappears much earlier.

7. The third branchial pouch swells out into an epithelial follicle, with many secondary evaginations. This becomes constricted off from the branchial gut, and the evaginations transform themselves into thymus-tissue, in the interior of which, however, the central epithelial follicle persists. The latter may be regarded as the homologue of the carotid body in the lizards.

8. The fourth and fifth branchial pouches develop jointly with the above-mentioned supra-pericardial evaginations from a lateral cæcum-shaped fold at the posterior end of the branchial gut (*recessus præcervicalis*), similarly to what is found to be the case in snakes. They soon become entirely constricted off from the branchial gut, and in this manner form a complex of three epithelial vesicles in connexion one with the other. Now, if their further development also takes place on the same lines as in the snakes, the two foremost of these vesicles, which represent the remnants of the fourth and fifth branchial pouches, should develop into thymus-tissue, while the third and hindermost should, on the contrary, remain in an epithelial condition. This, however, is not the case: all three retain an epithelial character, and are met with in this shape, even in much later developmental stages, between the aortic and pulmonary arches. They do not come into connexion with the thyroid.

9. The aorta develops from the artery of the fourth branchial arch, the pulmonary artery from that of the sixth. The fifth aortic arch, the rudiment of which arises between the fourth and fifth branchial pouches, very soon becomes aborted again, as I have also shown to be the case in snakes and lizards.

10. The observations here detailed confirm the theories as to the probable origin of the thymus and of the epithelial rudiments in the cervical region, which I arrived at in the anatomical investigation of young turtles, and to which I have already drawn attention in a previous memoir ("Beiträge zur Kenntnis der Halsgegend bei Reptilien: I. Anatomischer Teil," published in 'Bijdragen tot de Dierkunde, uitgegeven door het Genootschap Natura Artis Magistra te Amsterdam,' 1880).—*Anatomischer Anzeiger*, viii. Jahrg., nos. 23 and 24, October 10, 1893, pp. 801–803.

Observations on the Karyokinetic Phenomena in the Cells of the Blastoderm of Teleosteans. By MM. E. Bataillon and R. Kœhler.

In a previous communication we have described the results of our researches upon the extension of the blastoderm on the surface of

the ovum in the dace. We now propose to give an account of the curious phenomena which we have observed in the division of the cells of the blastoderm during the first days of the development of the embryo.

Method.—Before proceeding to the examination of our results, we must say a few words as to the method which we employed in order to determine their precise signification. The embryos, after being liberated from their shells, were fixed in Flemming's fluid, and the sections were treated in the following manner :—They were stained with borax methylene blue, then passed into water and very quickly into a strong aqueous solution of eosin, finally they were dehydrated and mounted in balsam.

Let us consider, for example, a transverse section from the dace a few days before being hatched (eight days after fertilization). Under a very slight magnifying-power we perceive, it may be in the nerve-centres or in the masses of mesoblast, blue spots, which form a contrast with the ground-colour of the section, which is distinctly of a reddish hue. Under a sufficiently high magnifying-power we discover that these blue spots are beautiful karyokinetic figures, the chromatin of which exhibits in all the stages a very delicate affinity for the methylene blue. The granulations of the resting nucleus are stained by the eosin : only one or two granules, which are clearly indicated, react like the chromatic portions of the figures of division, and may be regarded as nucleoli. Two important conclusions result from these observations :—

1. *Methylene blue is, under these conditions, a genuine reagent for chromatin in an active state.*

2. *With the exception of the nucleolus or nucleoli, the granulations of the resting nucleus have not the same reaction as the chromatin of division; their chemical composition must therefore undergo a change when they become constituent parts of the filament.*

We would remark that if the action of the eosin is too prolonged, or if the decoloration with alcohol is pushed too far, the blue tint will entirely disappear. Here we have a device of preparation. But since the results of this device are constant for the various stages, in numerous sections and in different tissues, it must correspond to a constant condition. The method indicated seems to us to be of value for the *technique* of cytology, which is, moreover, made up of devices.

Results.—Let us apply this method to the study of division in the first stages of development. During the whole of the first day the blastoderm-spheres, which multiply rapidly, exhibit all the stages of indirect division. In these cells we observe the rays of the asters extending from the periphery of the nucleus to the membrane throughout the whole mass of the protoplasm. These asters are either simple or double ; all the stages in the division of the centrosomes and their migration towards the poles are easily followed. Until the moment when the spindles appear the nuclei look like clear areolæ bounded by a delicate membrane ; not only are the few

granulations which they contain not coloured blue, but the paleness of the nuclear cavity contrasts strongly with the violaceous ground of the protoplasm, which, in this stage, seems to be impregnated in a diffuse fashion at once with the methylene blue and the eosin.

The division-spindles, of which the centrosomes alone have somewhat more colour, contain neither granules nor chromatic fibrils. It is necessary to examine at the end of the first day blastoderms with from ten to twelve rows of cells, in order to find, in certain cases of karyokinesis, a few extremely minute blue granules, which constitute the first equatorial plate. It is, moreover, at this stage that the protoplasm commences to exhibit the interesting peculiarities upon which we shall proceed to dwell.

From the thirty-sixth hour the methylene blue constantly reacts in the manner which we have indicated above.

Our double coloration therefore renders clearly evident the fact mentioned by MM. Henneguy and Sabatier, namely that the nuclei of the earliest embryonic stages are difficult to stain; but it has enabled us besides to recognize the appearance in the protoplasm of the cells of elements to which we attribute great importance, because they seem to us to give the key to this peculiarity. Between the twenty-fourth and the thirty-sixth hour, when only a few figures of division exhibit affinity for the methylene blue, this stain sharply distinguishes in the cellular protoplasm certain spherules, of which the tint is so much the deeper in proportion as their dimensions are reduced. With objectives of high power we perceive that the largest spherules differentiate in the interior of their paler mass smaller granules of very deep colour, which are marked out within the surrounding protoplasm. In neighbouring cells it may be observed that these granules, whether isolated or arranged in a series, are supported by the filaments of an aster: it seems that the latter serve to attract them, or, at least, to direct them towards the middle of the spindle, in order to furnish the elements of the equatorial plate. Henceforth all the cells in process of division will exhibit, contrary to what was seen in the first karyokineses, equatorial plates which are very distinct and of a vivid blue colour.

We do not think that the existence of these chromophilous granules, representing the earliest condition in which the chromatin substance manifests itself in the cells, has been mentioned before. In a memoir upon the development of bony fishes Miecz. von Kovalevsky represents the protoplasm of the cells of the blastoderm as containing granulations which may be compared with those which we are describing, but which are designated by him *vitelline granulations* ("granulations vitellines"). Owing to the fact that the investigations of this naturalist were conducted upon other material and by the aid of methods different from our own, it is impossible for us to discuss the interpretation which he gives to these elements. We would merely observe, and we insist upon this fact, that in our better preparations, where the chromophilous

granules are intensely stained by the blue colour, the vitellus is perfectly colourless, and that it is impossible to consider these granules as vitelline elements, since the methylene blue solely affects chromatin in an active condition, as we have already stated.

Conclusions.—1. *In the first stages of development in the dace the blastoderm-cells exhibit no individualized chromatin, and the karyokinetic figures are exclusively formed of achromatic elements.* This important fact furnishes support to the opinion which the most recent researches tend to make the prevalent one, namely that in the cell the essential *rôle* does not belong to the chromatin, as was formerly believed, but must rather be ascribed to the centrosomes.

2. *The chromatin at first exists in a diffused condition in the protoplasm, as certain authors have stated. It becomes differentiated and individualized in this protoplasm in the form of granulations which can be stained by means of reagents; then it becomes incorporated into the nuclei to constitute the equatorial plates which are absent in the first stages.*— *Comptes Rendus*, t. cxvii. no. 16, October 16, 1893, pp. 521–524.

On the Cerebral Nuclei of Myriopods.

By M. Joannes Chatin.

It is well known how much interest attaches at the present time to the study of the elements of the nervous system in Invertebrates. By putting into precise form the results thus obtained by zoological histology, and contrasting them with the facts revealed by histogeny, we shall succeed in elucidating and interpreting exactly the comparative structure of the nervous tissue, with respect to which so many points still remain obscure or imperfectly understood.

One of these points is the notion of the *cerebral nuclei*, *ganglionic nuclei*, &c., which have been stated to occur in the Articulata, and especially in the class Myriopoda, where, in the accounts of various investigations, mention has been made under this name of elements which are represented as formations of a special character and of high functional value. It is, however, only necessary to compare these descriptions in order to prove that they apply, in the respective cases, to different elements, the importance and independence of which henceforth become somewhat doubtful.

Since, therefore, it was imperative that the subject should be re-examined in a rigorous fashion, I undertook with this object a series of researches which were devoted especially to various species of the group Chilopoda (*Lithobius forficatus*, *Scolopendra morsitans*, *Scutigera coleoptrata*, &c.). I purposely chose these types because they had been mentioned as exhibiting the cerebral or ganglionic nuclei with exceptional distinctness.